

## Short communication

# Genetic and biologic characterization of *Toxoplasma gondii* isolates of cats from China

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**Abstract**

Cats are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete the environmentally resistant oocysts. In the present study, prevalence of *T. gondii* was determined in serum, feces, and tissues of 34 cats from People's Republic of China. Antibodies to *T. gondii* were assayed by the modified agglutination test and found in 27 of 34 (79.4%) cats with titers of 1:40 in one, 1:80 in one, 1:160 in three, 1:320 in three, 1:640 in eight, and 1:1280 or higher in 11 cats. *T. gondii* oocysts were not found in feces of any cat as ascertained by bioassay in mice. Tissues (brain, heart, and tongue) of 27 seropositive cats were pooled and bioassayed in mice (8 cats) or cats (19 cats). *T. gondii* was isolated from tissues of 17 of 27 seropositive cats. Genotyping of these 17 *T. gondii* isolates using polymorphisms at 10 nuclear markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and a new SAG2, and an apicoplast marker Apico revealed two genotypes. This is the first report of genetic typing of *T. gondii* isolates from cats from China.

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**Keywords:** *Toxoplasma gondii*; Cats; China; Feces; Antibodies; Genotyping

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**1. Introduction**

*Toxoplasma gondii* infections are widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988). Cats are important in the natural life cycle of *T. gondii* because they are the only hosts that can directly spread *T. gondii* in the environment. Additionally, in People's Republic of China (PRC) cat meat is sold for human consumption, which can be a

potential source for *T. gondii* transmission. However, little is known of the prevalence of viable *T. gondii* in cats from PRC even though antibodies to *T. gondii* in these animals were reported in local Chinese journals (see Table 1). The objectives of the present study were to determine the prevalence of *T. gondii* in cats from PRC and characterize isolates of *T. gondii* from these cats.

**2. Materials and methods****2.1. Naturally infected cats**

Samples from 34 cats were received in four batches (A–D) of 6, 10, 10, and 8 cats from January–September,

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Table 1  
Prevalence of *T. gondii* antibodies in pet cats in People's Republic of China

Geographical location	Prevalence rate		Detection methods	Reference
	Total number of cats	Percent positive		
Beijing	128	14.1	ELISA <sup>a</sup>	Yu et al. (2006)
Guangzhou	114	23.7	ELISA	Chen et al. (2005)
Hebei	75	57.3	ELISA	Yuan et al. (2004)
Hubei	105	31.4	ELISA	Chen (2001)
Shanghai	142	38.1	IHA <sup>b</sup>	Lu et al. (1997)
Shandong	200	46.0	IHA	Fu et al. (1995)
Guangzhou	34	79.4	MAT	Present study

<sup>a</sup> Enzyme linked immunosorbent assay.

<sup>b</sup> Indirect hemagglutination test.

2006, respectively (Table 2). The clinically healthy cats of local breeds were bought from Changban Free Market, Guangzhou, Guangdong Province, PRC. The cats were slaughtered humanely according to PRC laws for slaughtering of food animals. From each cat, serum, brain, tongue, and heart, and feces were obtained for *T. gondii* examination. Samples were transported by air from PRC to the Animal Parasitic Diseases Laboratory (APDL), U.S. Department of Agriculture (USDA) Beltsville, MD, where all *T. gondii* evaluations were performed using protocols approved by the USDA. Four to 10 days elapsed between shipping from PRC and receipt at APDL.

## 2.2. Serologic examination

Sera from cats were diluted two-fold starting at 1:10 to 1280 and assayed for *T. gondii* antibodies with the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987).

## 2.3. Fecal examination

Feces (2–10 g) collected from the rectum of 26 cats were floated in sugar solution and a drop from the meniscus was examined microscopically between cover slip and glass slide. Fecal floats were sedimented in

water, and aerated in 2% sulfuric acid on a shaker at 22 °C for 1 week and then bioassayed in mice orally (Dubey and Beattie, 1988).

## 2.4. Bioassay of tissues for *T. gondii*

Tissues of all 27 cats with MAT titers of 1:40 were bioassayed for *T. gondii*; tissues from eight cats (batch B) were bioassayed in mice and tissues from 19 cats were bioassayed in cats (Table 2). For bioassays in mice or cats, tongue (25 g), brain (25 g), and heart (25 g) of each cat were pooled. For bioassays in cats, pooled feline tissues were fed to 19 *T. gondii*-free cats. Feces of cats were examined for shedding of *T. gondii* oocysts 3–14 days after feeding feline tissues as previously described (Dubey et al., 2004). Fecal floats from these cats were bioassayed orally in mice (Dubey and Beattie, 1988).

For mouse bioassay, pooled tissues from each of the eight cats in batch B were homogenized in five volumes (w/v) of saline, mixed with five volumes of acidic pepsin and the mixture incubated in a shaker water bath for 1 h at 37 °C. The digest was centrifuged, neutralized, mixed with antibiotics, and the homogenate was inoculated subcutaneously in to four mice for each cat (Dubey, 1998). The mice used were Swiss Webster albino females obtained from Taconic Farms, Germantown, New York. Mice were examined for viable *T.*

Table 2  
Isolation of *T. gondii* from cats from PRC

Batch no (Expt. No.)	No. of cats	No. seropositive	No. bioassayed		<i>T. gondii</i> isolates
			In cats	In mice	
A (TX 225)	6	5	5	0	3
B (TX 244)	10	8	0	8	2
C (TX 251)	10	8	8	0	6
D (TX 269)	8	6	6	0	6
Total	34	27	19	8	17

*gondii* parasites as described (Dubey et al., 2004). Mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were demonstrable in their tissues.

### 2.5. Genetic characterization

*T. gondii* DNA was extracted from the tissues of infected mice from each group and strain typing was initially performed using PCR-RFLP genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 (Dubey et al., 2006). In brief, the target DNA sequences were amplified by multiplex PCR using external primers for all five markers. Multiplex PCR amplified products were then used for nested PCR with internal primers for each marker separately. Nested PCR products were treated with restriction enzymes and resolved in agarose gel by electrophoresis to reveal the RFLP patterns of the isolates. These five markers allow us to quickly characterize all samples and to identify potential mixed infection in cats. One or two representative DNA extracts from mice infected with the same cat sample were genotyped with six additional genetic markers including c22-8, c29-2, L358, PK1, a new SAG2 and Apico to further identify isolates with high resolution (Dubey et al., 2007; Su et al., 2006) by the same method described above. Allele types for all isolates were determined based on the RFLP patterns of six reference strains including RH88, PTG, CTG, COUGAR, MAS

and TgCatBr5 (Su et al., 2006). These reference strains allow us to capture all known alleles for each marker and to identify potential unique alleles in new samples.

### 3. Results

Antibodies to *T. gondii* were found in 27 of 34 (79.4%) cats with titers of 1:40 in one, 1:80 in one, 1:160 in three, 1:320 in three, 1:640 in eight, and 1:1280 or higher in 11 cats. *T. gondii* oocysts were not found (both microscopically and by bioassay) in feces of any of the naturally exposed cats from China.

In total, *T. gondii* was isolated from tissues of 17 (15 by bioassay in cats and 2 by bioassay in mice) of 27 seropositive cats (Table 2). Fifteen of the 19 cats fed feline tissues shed oocysts; these oocysts were bioassayed in mice. Mice inoculated orally with oocysts of nine of the 15 cats became ill or died within 12 days p.i. but mice inoculated with tachyzoites of these isolates became infected with *T. gondii* but remained asymptomatic, indicating that virulence was stage dependent. All mice inoculated with tissue digests of cats in batch B became sick the next day and in spite of antibiotic therapy; 28 of 40 died within 4 days p.i., and were discarded; Tissue cysts were found in four of five mice that survived after inoculation with tissues of two cats. The *T. gondii* isolates identified in this study were designated as TgCtPRC 1–17 (Tables 2 and 3).

Table 3

Isolation of viable *T. gondii* from tissues of PRC cats by bioassays in experimental cats

PRC donor cats		Recipient cats		Oocysts fed <sup>a</sup>	Tachyzoites inoculated	Strain designation
No.	Tx	No.	Oocysts shed			
1	320	235	+	9 <sup>a</sup> , 12	26, 29	TgCtPRC 1
4	640	272	+	4, 4	60 <sup>b</sup> , 60 <sup>b</sup>	TgCtPRC 2
6	640	240	+	7, 7	30,	TgCtPRC 3
Tx 251						
20	320	284	+	60, 60		TgCtPRC 6
21	1280	286	+	4, 4	56 <sup>c</sup> , 56 <sup>c</sup>	TgCtPRC 7
23	1280	285	+	7, 8	53 <sup>c</sup> , 53 <sup>c</sup>	TgCtPRC 8
24	1280	279	+	4, 4	56 <sup>c</sup> , 56 <sup>c</sup>	TgCtPRC 9
25	640	289	+	60, 60		TgCtPRC 10
26	640	280	+	4, 4	56 <sup>c</sup> , 56 <sup>c</sup>	TgCtPRC 11
TX 269						
43	160	323	+	4, 5	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC12
45	320	338	+	4, 5	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC 13
46	1280	311	+	5, 40 <sup>c</sup>	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC 14
47	1280	331	+	5, 40 <sup>c</sup>	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC 15
48	160	313	+	4, 40 <sup>c</sup>	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC 16
49	640	340	+	5, 40 <sup>c</sup>	59 <sup>c</sup> , 59 <sup>c</sup>	TgCtPRC 17

<sup>a</sup> Two mice were feed oocysts, day indicated is p.i. mice killed.

<sup>b</sup> Two mice were inoculated with mesenteric lymph node homogenate of mice fed oocysts.

<sup>c</sup> Mice were asymptomatic and killed on indicated days.

Table 4  
Isolation of viable *T. gondii* from tissues of PRC cats by bioassays in mice

PRC donor cats		Bioassay in mice <sup>a</sup>		<i>T. gondii</i> positive/no. of mice survived	Isolate designation
No. Tx 244	MAT				
7	1280	D2, D2, D2, D2	Discarded	NA	TgCtPRC 4
8	80	D1, S, S, S		3/3	
11	160	D1, D2, D3, S		1/1	
12	40	D2, D2, D3, D4	Discarded	NA	TgCtPRC 5
13	1280	D2, S, S, S		0/3	
14	1280	D3, S, S, S		0/3	
15	640	D2, D3, D3, S		0/1	
16	1280	D2, D2, D3, D4	Discarded	NA	

D = died; S = survived and killed on day 41 p.i.; NA = not applicable.

<sup>a</sup> Four mice were inoculated with homogenate of feline tissues.

Genotyping of these 17 isolates using polymorphisms at the SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2, and an apico plast marker Apico revealed two genotypes, with isolates TgCtPRC1 and 3 belonging to one genotype and the rest 15 isolates to another genotype. Both genotypes are different from Type I, II and III lineages that predominate in North America and Europe.

#### 4. Discussion

In the present study, *T. gondii* antibodies were found in 79.4% of 34 cats tested. This is the highest seroprevalence reported among cats surveyed in PRC. Data from previous surveys are tabulated in Table 1. It is clear that the serum positivity varied widely among these studies, these differences maybe due to the serological tests used. More is known of the efficacy of the MAT for the diagnosis of *T. gondii* antibodies in cats than other tests.

In previous studies the results were based on the indirect hemagglutination test (IHAT) or the ELISA. The IHAT has been found to be insensitive for serodiagnosis in experimentally infected cats (Dubey and Thulliez, 1989; Dubey et al., 1995). The sensitivity and the specificity of the ELISA are based on the reagents and standardization of the tests. The MAT test was found to be very efficacious in cats based on comparison of the serologic data and the bioassays of tissues of naturally infected cats (Dubey et al., 2004; Pena et al., 2006).

In the present study, *T. gondii* oocysts were not found in feces of any of the 34 cats. The most likely reason for this result is the high prevalence of antibodies to *T. gondii*. In experimental infections cats shed oocysts for only 1–2 weeks and they were seronegative during the period of oocyst shedding. Thus, by the time the cats become seropositive they have already shed oocysts. In natural environment, *T. gondii* oocysts were found in 1% of cats at any given time (Dubey and Beattie, 1988).

Table 5  
Genotyping of *T. gondii* isolates from cats from PRC

Isolate ID	SAG1	SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	New SAG2	Apico
TgCtPRC1	I	I	III	I	III	II	I	III	III	I	I
TgCtPRC2	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC3	I	I	III	I	III	II	I	III	III	I	I
TgCtPRC6	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC7	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC8	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC9	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC10	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC11	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC12	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC13	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC14	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC15	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC16	u-1	II	III	III	II	II	III	II	II	II	I
TgCtPRC17	u-1	II	III	III	II	II	III	II	II	II	I

The isolation of *T. gondii* from tissues of PRC cats was more efficient by bioassay in cats than mice (Tables 4 and 5). Fifteen of 19 (78.9%) cats fed feline tissues shed *T. gondii* oocysts whereas *T. gondii* was isolated from only two of eight cats by bioassay in mice. Difference in these results was in part due to autolysis of tissues bioassayed in mice; most mice died of bacterial infection.

Genotyping of the 17 cat isolates of *T. gondii* identified only two genotypes, indicating limited diversity of the parasite in the region surveyed. As the cats-studies here were obtained from free market and their sources are unclear, it is difficult to predict the overall diversity of the parasite. However, only two genotypes were identified in this study suggesting that only few *T. gondii* genotypes may circulate in PRC. This is in sharp contrast to South America where a variety of diverse parasite lineages are transmitted in the environment (Dubey et al., 2005, 2006, 2007; Lehmann et al., 2006).

In summary, this study showed that high percentage of cats in PCR is infected with *T. gondii*, which may serve as an important source for transmission of the parasite. The results are of public health significance because cat meat is consumed by humans in China, and thus humans can become infected directly from cats, in addition to the oocysts excreted by infected cats.

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